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ACUTE EFFECTS OF ROAD SALTS AND ASSOCIATED CYANIDE COMPOUNDS ON THE EARLY LIFE STAGES OF THE UNIONID MUSSEL *VILLOSA IRIS*

TAMARA J. PANDOLFO,*† W. GREGORY COPE,‡ GEORGE B. YOUNG,§ JESS W. JONES,||
DAN HUA,# and SUSAN F. LINGENFELSER††
†North Carolina Cooperative Fish and Wildlife Research Unit, Department of Biology, North Carolina State University, Raleigh, North Carolina, USA
‡Department of Environmental and Molecular Toxicology, North Carolina State University, Raleigh, North Carolina, USA
§Virginia Department of Transportation, Bristol, Virginia USA
||United States Fish and Wildlife Service, Department of Fish and Wildlife Conservation, Virginia Tech University, Blacksburg, Virginia
#Department of Fish and Wildlife Conservation, Virginia Tech University, Blacksburg, Virginia, USA
††Virginia Ecological Services Field Office, United States Fish and Wildlife Service, Gloucester, Virginia

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Abstract—The toxicity of cyanide to the early life stages of freshwater mussels (order Unionida) has remained unexplored. Cyanide is known to be acutely toxic to other aquatic organisms. Cyanide-containing compounds, such as sodium ferrocyanide and ferric ferrocyanide, are commonly added to road deicing salts as anticaking agents. The purpose of the present study was to assess the acute toxicity of three cyanide compounds (sodium cyanide, sodium ferrocyanide, and ferric ferrocyanide), two road salts containing cyanide anticaking agents (Morton and Cargill brands), a brine deicing solution (Liquidow brand), and a reference salt (sodium chloride) on glochidia (larvae) and juveniles of the freshwater mussel *Villosa iris*. Sodium ferrocyanide and ferric ferrocyanide were not acutely toxic to glochidia and juvenile mussels at concentrations up to 1,000 mg/L and 100 mg/L, respectively. Lowest observed effect concentrations (LOECs) for these two chemicals ranged from 10 to >1,000 mg/L. Sodium cyanide was acutely toxic to juvenile mussels, with a 96-h median effective concentration (EC50) of 1.10 mg/L, although glochidia tolerated concentrations up to 10 mg/L. The EC50s for sodium chloride, Liquidow brine, Morton road salt, and Cargill road salt were not significantly different for tests within the same life stage and test duration (range, 1.66–4.92 g/L). These results indicate that cyanide-containing anticaking agents do not exacerbate the toxicity of road salts, but that the use of road salts and brine solutions for deicing or dust control on roads may warrant further investigation. Environ. Toxicol. Chem. 2012;31:1801–1806. © 2012 SETAC

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INTRODUCTION

Freshwater mussels of the bivalve order Unionida are one of the most rapidly declining faunal groups globally, but especially in North America where approximately 70% of the nearly 300 mussel species are extinct or vulnerable to extinction [1–4]. This decline has been attributed to a range of anthropogenic sources, including habitat degradation, land use change, and pollution [5,6].

The sensitivity of freshwater mussels to a range of contaminants has been widely tested, and results of these studies indicate that the early life stages of freshwater mussels are more sensitive to some contaminants, such as copper and ammonia, than other commonly tested aquatic organisms [6–8]. The toxicity of cyanide to freshwater mussels has remained unexplored, although cyanide is known to be acutely toxic to other aquatic organisms [9–12].

The toxicity of cyanide is a relevant concern due to the inclusion of cyanide-containing chemicals in road salts. These chemicals, such as sodium ferrocyanide and ferric ferrocyanide, serve as anticaking agents for the sodium chloride road salts [13]. Road salts are common in the environment, with 12.2 and 9.3 million tons of road-deicing salt sold in the United States in 2008 and 2009, respectively [14].

In addition to potential hazards from cyanide, road-deicing salts have the ability to salinize freshwaters. The toxicity of salt to freshwater mussels is well established [6,15–17]. Chloride concentrations in freshwater peak in urban areas during the winter [18,19] and chloride concentrations in some streams already exceed the recommended limit for the protection of freshwater organisms [18]. The aim of the present study was to determine the acute toxicity of cyanide compounds and cyanide-containing road salts to a freshwater mussel and to determine whether cyanide compounds exacerbate the toxicity of road salts.

MATERIALS AND METHODS

Test chemicals

Three cyanide compounds were tested: sodium cyanide (CAS 143-33-9; Fisher Scientific), sodium ferrocyanide (CAS 13601-19-9; Spectrum Chemical Manufacturing), and ferric ferrocyanide (CAS 14038-43-8; Acros Organics). Two cyanide-containing road salts (Morton and Cargill brands), and a Liquidow brine solution (Sicalco) were supplied by the Virginia Department of Transportation for testing. Cargill brand road salt contains 95.8 to 99.8% sodium chloride and 0.005 to 0.01% sodium ferrocyanide. Morton brand road salt contains >95% sodium chloride and <0.01% sodium ferrocyanide. Liquidow brine solution contains 28 to 42% calcium chloride, 0 to 3% potassium chloride, and 0 to 2% sodium chloride. Sodium chloride (CAS 7647-14-5; Fisher Scientific) was used as a reference salt for the Cargill and Morton road salts. Total cyanide concentrations in samples of test water were analyzed for verification of exposure concentrations according to U.S.

* To whom correspondence may be addressed (tjpandol@ncsu.edu).
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Environmental Protection Agency (U.S. EPA) Method 335.4 by Environmental Conservation Laboratories (Cary, NC, USA). A rigorous quality assurance protocol was followed during analysis of total cyanide in test solutions. Quality assurance included blanks, laboratory control samples, matrix spikes, and matrix spike duplicates. The blanks were uncontaminated; no target analyte was detected above the method reporting limit of 0.005 mg/L. The relative percent difference of duplicate samples averaged 2%. Overall, percent recovery of spiked samples averaged 106% and ranged from 105 to 108%. All laboratory control sample percent recoveries were within the certified range (mean, 105%; range, 90–110%).

Test organisms

The freshwater mussel *Villosa iris* (rainbow) was used for all toxicity tests. All mussels were supplied by the Freshwater Mollusk Conservation Center at Virginia Tech University. Glochidia were obtained from three gravid adult mussels collected from Indian Creek in Tazewell, Virginia, USA. Juvenile mussels were propagated using *Ambloplites rupestris* (rock bass) for transformation. One-day-old juvenile mussels were placed in small holding chambers that were held in a bucket rearing system [20]. Approximately 12 L of natural pond water at the Freshwater Mollusk Conservation Center in Blacksburg, Virginia, USA, was filtered through a 5-μm nylon filter bag and placed in the bucket. Water in the bucket was completely replaced every 5 to 7 d. Juvenile mussels were reared on a commercial shellfish algal diet (Reed Mariculture) and fed continuously with an automated feeding control system. Cell density in the buckets ranged daily from 30,000 to 50,000 cells/ml. Water temperature in the buckets was held constant at 21°C, pH ranged from 8.0 to 8.5, and dissolved oxygen averaged 7.5 mg/L. Juveniles were reared to 300 to 500 μm in length and one month of age before being shipped to North Carolina State University for testing; the average length of mussels tested was 465 μm.

Glochidia assessment

Glochidia were <24 h old at the start of each test, and they were acclimated to reconstituted hard water and the test temperature of 20°C for 2 h before the beginning of the experiments [15,21]. Tests were 48-h nonaerated static experiments conducted according to the American Society of Testing and Materials guidelines for glochidia [15]. Survival was assessed at 24 h for a subsample of approximately 50 of the 150 glochidia in each of three replicates per treatment. A saturated sodium chloride solution was used to stimulate a shell-closure response; glochidia that were closed before adding the saturated sodium chloride and those that did not respond to the salt addition were considered nonviable. Salt closure responses were documented with an Olympus SZ61 microscope (Olympus America) and QCapture Pro 5.1 digital photographic software (Quantitative Imaging). The average control survival in glochidia tests was 87.9% (range, 82.2–95.6%) at the 24-h time point and 87.1% (range, 80.5–92.4%) at the 48-h time point. Results of glochidia tests with control survival of >80% are presented; not all tests met the 90% control survival guideline recommended by the American Society of Testing and Materials [15]. Control survival at 48 h in the Liquidow brine solution test was <80%; therefore, those results are not included in this manuscript.

Juvenile assessment

Juvenile mussels were acclimated to reconstituted hard water and the test temperature of 20°C for 2 d before the start of experiments [21]. Experiments were 96-h nonaerated static renewal tests conducted according to the *Standard Guide for Conducting Laboratory Toxicity Tests with Freshwater Mussels*, with 90% water and chemical renewal at 48 h [15]. Each one of three replicates had seven mussels per treatment, and each one of three replicates had 10 mussels in controls. Survival was assessed at 48 and 96 h using an Olympus SZ61 microscope (Olympus America) to detect foot movement outside the shell, foot movement within the shell, or the presence of a heart beat. Control survival for 48-h juvenile assessments averaged 96.1% (range, 83.3–100%) and averaged 90.6% (range, 75.7–100%) in 96-h assessments. Control survival was <90% at the 96-h assessment in the sodium chloride (75.7%) and Cargill road salt (79.1%) tests, and at the 48- and 96-h assessments in the Liquidow brine (83.3%) test. These results have been included in this manuscript, but they are denoted as having control survival <90%.

Water chemistry

Water-quality conditions, including alkalinity, hardness, conductivity, pH, temperature, and dissolved oxygen were measured at the start of each test and again at 48 h. Alkalinity and hardness were measured by titrametric procedures with standard methods [22]. Conductivity, temperature, pH, and dissolved oxygen were measured with a calibrated meter (YSI model 556 MPS multi-probe; Yellow Springs Instrument). Mean water-quality conditions among all tests were the following: 107.3 mg $CaCO_3$/L alkalinity (range, 104–110 mg $CaCO_3$/L); 162.5 mg $CaCO_3$/L hardness (range, 156–170 mg $CaCO_3$/L); 555.2 μs/cm conductivity (range, 516–613 μs/cm); 8.29 pH (range, 7.38–8.61); 20.4°C temperature (range, 19.4–21.0°C); and 8.56 mg/L dissolved oxygen (range, 7.65–9.08 mg/L).

Statistical analysis

Survival data from both glochidia and juvenile tests were used to generate median effective concentrations (EC50s) and 95% confidence intervals using the trimmed Spearman–Karber method with ToxCalc v.5.0.26 toxicity data analysis software (Tidepool Scientfic). The EC50 was defined as the chemical concentration that elicited an adverse response in 50% of the exposed population. For glochidia, the adverse response was indicated by failure to close their valves in response to salt stimulus. In juvenile tests, the adverse response was lack of foot movement or heartbeat. If 95% confidence intervals did not overlap, EC50s were considered significantly different. As an additional measure of toxicity, the no observed effect concentration (NOEC) and lowest observed effect concentration (LOEC) for each test were determined using Dunnett's multiple comparison and the Bonferroni adjusted *t* test with Comprehensive Environmental Toxicity Information Software (CETIS) v1.8.0.12 (Tidepool Scientific).

RESULTS AND DISCUSSION

Cyanide verification

Percent of expected total cyanide measured in samples of Cargill road salt, Morton road salt, ferric ferrocyanide, sodium ferrocyanide, and sodium cyanide ranged from 8.5 to 111.8% (Table 1). The wide range among nominal and measured concentrations is largely related to the availability of cyanide within the different compounds. Measured sodium cyanide concentrations ranged from 92.3 to 97.9% of expected nominal concentrations, indicating high cyanide availability. Measured sodium ferrocyanide concentrations were 65.2 to 111.8% of expected nominal cyanide, with the lowest measured cyanide at

Table 1. Cyanide-containing test chemicals, target exposure concentrations, proportion composed of cyanide (CN), and percentage of expected CN from analysis for total cyanide in test chemicals

Chemical	Test concentration (mg/L)	CN in chemical (% w/w)	Expected CN (mg/L)	Actual CN (mg/L)	Percentage of expected CN
Cargill road salt	4000	0.0000322	0.129	0.011	8.5
Cargill road salt	8000	0.0000322	0.258	0.022	8.5
Morton road salt	2000	0.0000322	0.064	0.046	71.4
Morton road salt	4000	0.0000322	0.129	0.1	77.6
Ferric ferrocyanide	10	0.545	5.45	3.5	64.2
Ferric ferrocyanide	100	0.545	54.5	7.8	14.3
Ferric ferrocyanide	1000	0.545	545.0	54	9.9
Sodium ferrocyanide	10	0.322	3.22	3.6	111.8
Sodium ferrocyanide	100	0.322	32.2	33	102.5
Sodium ferrocyanide	1000	0.322	322.0	210	65.2
Sodium cyanide	0.1	0.531	0.053	0.049	92.3
Sodium cyanide	1	0.531	0.531	0.52	97.9
Sodium cyanide	10	0.531	5.310	5	94.2

the greatest concentration. Ferric ferrocyanide measured 9.9 to 64.2% of expected nominal cyanide concentrations, indicating low cyanide availability. Ferric ferrocyanide is highly insoluble in water, and its cyanide groups are tightly bound in iron complexes; therefore, a low availability was expected. Total cyanide measurements in the road salts varied by brand, with Cargill road salt having only 8.5% of expected nominal total cyanide and Morton road salt having 71.4 to 77.6%. Because the chemical components of the road salts are similar, the differences in measured cyanide were unexpected. However, it is possible that the distribution of the <0.01% sodium ferrocyanide within the sodium chloride was not homogenous, allowing for apparent differences in cyanide measurements.

Cyanide toxicity

The present study was the first to attempt to determine the toxicity of cyanide compounds to unionid mussels. Sodium ferrocyanide and ferric ferrocyanide were not acutely toxic to glochidia or juvenile freshwater mussels at concentrations up to 1,000 mg/L and 100 mg/L, respectively (Table 2). The NOECs and LOECs for glochidia in the 24-h sodium ferrocyanide test were 100 and 200 mg/L, respectively, and at 48 h these values increased to 1,000 and >1,000 mg/L (Table 3). Ferric ferrocyanide elicited a NOEC and LOEC for glochidia of 100 and >100 mg/L at 24 h and 10 and 100 mg/L at 48 h, respectively. In juvenile tests, both the 48- and 96-h NOECs and LOECs for sodium ferrocyanide were 1.0 and 10 mg/L. At 48 h, ferric ferrocyanide elicited a NOEC and LOEC of 100 and >100 mg/L in juveniles, and at 96 h these values decreased to 10 and 100 mg/L.

Sodium cyanide was acutely toxic to juvenile freshwater mussels. A 48-h EC50 of 4.81 mg/L and a 96-h EC50 of 1.10 mg/L were generated for juvenile *V. iris*. Glochidia were more tolerant of sodium cyanide exposure, with survival at concentrations up to 10 mg/L. The NOECs and LOECs for glochidia exposed to sodium cyanide for 24 h were 1.0 and 10 mg/L, respectively, and at 48 h these values were 10 and >10 mg/L, respectively. These values are not very different from the NOEC and LOEC for juveniles at 48 h (1.0 and 10 mg/L, respectively), but they are much higher than the NOEC and LOEC for juveniles at 96 h (<0.001 and 0.001 mg/L, respectively).

The finding that juvenile *V. iris* were more sensitive to sodium cyanide than glochidia was not unexpected because the target sites for toxicity in freshwater organisms are sites such as gills, where gas exchange and osmoregulation occur [23]. Freshwater mussel glochidia are obligate parasites that must attach to a host fish in order to metamorphose into the juvenile life stage. During metamorphosis on the host fish, there is a reorganization of the body structure, including the development of initial gill structures [23]. The glochidia that were used in the present study were <24 h old and had not parasitized host fish; therefore, their gills had not begun to form [23]. Differences in cyanide toxicity among life stages are common, particularly in fish [10]. Eggs tend to be more tolerant to cyanide

Table 2. Median effective concentrations (EC50s) for glochidia and juvenile *Villosa iris* exposed to seven cyanide- and salt-containing chemicals[a]

		Chemical						
Life stage	Time point (h)	Sodium chloride (g/L)	Liquidow brine (g/L)	Morton road salt (g/L)	Cargill road salt (g/L)	Sodium cyanide (mg/L)	Sodium ferrocyanide (mg/L)	Ferric ferrocyanide (mg/L)
Glochidia	24	1.70 (0.94–3.08)	2.56 (2.41–2.71)	2.29 (0.96–5.43)	1.84 (0.96–3.52)	>10	>1,000	>100
	48	1.78 (1.03–3.10)	ND	2.22 (1.20–4.11)	2.09 (0.88–4.93)	>10	>1,000	>100
Juveniles	48	2.30 (1.15–4.58)	2.75* (1.70–4.40)	4.92 (3.33–7.25)	4.12 (2.49–6.81)	4.81 (1.84–12.58)	>1,000	>100
	96	1.66* (0.89–3.10)	2.40* (1.60–3.60)	3.42 (1.74–6.72)	3.85* (2.29–6.47)	1.10 (0.22–5.40)	>1,000	>100

[a] Numbers in parentheses represent the 95% confidence interval.
* Juvenile control survival < 90%.
ND = not detected.

Table 3. No observed effect concentrations (NOECs) and lowest observed effect concentrations (LOECs) for glochidia and juvenile *Villosa iris* exposed to seven cyanide- and salt-containing chemicals

Sodium chloride (g/L)		Liquidow brine (g/L)		Morton road salt (g/L)		Cargill road salt (g/L)		Sodium cyanide (mg/L)		Sodium ferrocyanide (mg/L)		Ferric ferrocyanide (mg/L)	
NOEC	LOEC	NOEC	LOEC	NOEC	LOEC	NOEC	LOEC	NOEC	LOEC	NOEC	LOEC	NOEC	LOEC
0.5	1.0	1.0	2.0	<0.25	0.25	0.25	0.5	1.0	10	100	200	100	>100
0.5	1.0	ND	ND	<0.25	0.25	<0.25	0.25	10	>10	1000	>1000	10	100
1.0	2.0	1.25	2.5	2.0	4.0	2.0	4.0	1.0	10	1.0	10	100	>100
2.0	4.0	1.25	2.5	2.0	4.0	4.0	8.0	<0.001	0.001	1.0	10	10	100

ND = not detected.

than juvenile fishes [10], which is similar to the pattern that was observed with the freshwater mussel in the present study.

Cyanide toxicity depends largely on its structure [24,25]. A measure of total cyanides includes all free, simple, and complex cyanides that are released by acidification or digestion of samples [12], but free cyanide is the most biologically relevant measure of cyanide. Free cyanide (molecular HCN and CN^{-}) is responsible for the majority of cyanide toxicity in aquatic systems. Molecular HCN is the dominant form of free cyanide in waters with pH ≤ 9.2, and the toxicity of simple cyanides, such as sodium cyanide, is not affected in waters with pH < 8.3 [9–11].

The toxicity of cyanide to the freshwater mussel tested in the present study is similar to cyanide toxicity in other aquatic organisms. When EC50s are expressed as exposure to CN, juvenile *V. iris* have a 96-h EC50 of 0.61 mg CN/L and a 48-h EC50 of 2.46 mg CN/L (Table 4). Freshwater fish are among the most sensitive of aquatic organisms to cyanide exposure [11]. In a review of the acute and chronic toxicity of cyanide, Gensemer et al. [12] determined that the geometric mean lethal concentration (LC50) among fish species ranged from 0.059 to 0.33 mg CN/L, with *Oncorhynchus mykiss* being the most sensitive. Similarly, others found that 0.05 to 0.2 mg CN/L caused mortality in sensitive fish species, and concentrations >0.2 mg CN/L were lethal to the majority of fish species over time periods ranging from 96 to 288 h [9–11,26,27]. The EC50s determined for juvenile *V. iris* in the present study demonstrate a greater cyanide tolerance in at least one species of freshwater mussel than among most fish species.

Invertebrates tend to be more tolerant of cyanide exposure, although they exhibit a range of sensitivities [10–12]. The crustaceans *Daphnia pulex* and *D. magna* had 96-h LC50s of 0.083 to 0.090 mg CN/L [12]. Aquatic insects are more tolerant: the water scorpion *Ranatra* spp. has a 96-h LC50 of 0.228 mg CN/L, and the midge *Tanytarsus dissimilis* has a 96-h LC50 of 2.49 mg CN/L [12]. The snail *Helisoma trivolvis* has a 96-h LC50 >50 mg CN/L [12], which exceeds the resistance demonstrated by *V. iris* in the present study. Based on results from toxicity studies such as these, the U.S. EPA established an ambient water quality criterion for cyanides of 0.022 mg/L to protect freshwater aquatic organisms against acute exposures [27], and the Canadian Council of Ministers of the Environment has set a limit of 0.005 mg/L of free cyanide to protect aquatic life [28]. These criteria appear to be adequately protective of freshwater mussels with tolerances similar to those of *V. iris*.

Salt toxicity

The results of our toxicity tests with salts indicate that road salts are not more toxic than reference salt (Table 2). The sodium ferrocyanide component in the road salts did not increase their toxicity to the freshwater mussel *V. iris*, and the two brands of road salt did not have significantly different toxicities despite their differences in measured cyanide content. This supports the finding that the sodium ferrocyanide component in the road salts did not exacerbate the toxicity of the sodium chloride in the road salts. Liquidow brine elicited a 24-h EC50 of 2.56 g/L for *V. iris* glochidia. Sodium chloride elicited a 24-h EC50 of 1.70 g/L and a 48-h EC50 of 1.78 g/L for glochidia. Morton road salt exposure resulted in a 24-h EC50 of 2.29 g/L and a 48-h EC50 of 2.22 g/L, and Cargill road salt exposure resulted in a 24-h EC50 of 1.84 g/L and a 48-h EC50 of 2.09 g/L. Glochidia EC50s for Liquidow brine, sodium chloride, Morton road salt, and Cargill road salt were not significantly different based on overlapping 95% confidence intervals. Although the EC50s for these chemicals did not differ, the LOECs for these chemicals indicated potential differences at the lower ranges of exposure. The 24-h LOECs for sodium chloride, Liquidow Brine, Morton road salt, and Cargill road salt were 1.0, 2.0, 0.25, and 0.5 g/L respectively.

Table 4. Median effective concentrations (EC50s) expressed as total chemical and total chemical as cyanide for glochida and juvenile *Villosa iris* exposed to three cyanide-containing chemicals[a]

Life stage	Time point (h)	Sodium cyanide (mg/L)	Sodium cyanide as CN (mg/L)	Sodium ferrocyanide (mg/L)	Sodium ferrocyanide as CN (mg/L)	Ferric ferrocyanide (mg/L)	Ferric ferrocyanide as CN (mg/L)
Glochidia	24	>10	>5	>1,000	>210	>100	>7.8
	48	>10	>5	>1,000	>210	>100	>7.8
Juveniles	48	4.81 (1.84–12.58)	2.46 (0.21–29.0)	>1,000	>210	>100	>7.8
	96	1.10 (0.22–5.40)	0.61 (0.02–21.6)	>1,000	>210	>100	>7.8

(Chemical columns: Sodium cyanide through Ferric ferrocyanide as CN.)

[a] Numbers in parentheses represent the 95% confidence interval.
CN = cyanide.

In juvenile *V. iris* tests, Liquidow brine elicited a 48-h EC50 of 2.75 g/L and a 96-h EC50 of 2.40 g/L. The 48-h EC50 for juveniles exposed to sodium chloride was 2.30 g/L, and at 96 h the EC50 was 1.66 g/L. Morton road salt elicited a 48-h EC50 of 4.92 g/L and a 96-h EC50 of 3.42 g/L, and Cargill road salt elicited a 48-h EC50 of 4.12 g/L and a 96-h EC50 of 3.85 g/L. As with the glochidia results, juvenile EC50s for Liquidow brine, sodium chloride, Morton road salt, and Cargill road salt were not significantly different within a test duration (i.e., 48 or 96 h). The 96-h LOECs for these tests were somewhat different, with sodium chloride, Liquidow brine, Morton road salt, and Cargill road salt eliciting LOECs of 4.0, 2.5, 4.0, and 8.0 g/L, respectively.

In the only significant differences found among the salts tested, the EC50 from the 48-h Morton road salt juvenile test was significantly greater than the juvenile 96-h sodium chloride, glochidia 24- and 48-h sodium chloride, and glochidia 24-h Liquidow brine EC50s. However, these differences are not comparable among life stage and test duration and therefore have little biological relevance. No differences were found between the sodium chloride and Liquidow brine solution, although calcium chloride is generally more acutely toxic to aquatic organisms than sodium chloride [29,30].

The sodium chloride toxicity values for *V. iris* from the present study are comparable to those for salt toxicity in other freshwater mussel species. Glochidia of four species (*Lampsilis siliquoidea*, *Lampsilis cardium*, *Lampsilis fasciola*, and *Epioblasma torulosa rangiana*) had 24-h EC50s ranging from 0.186 to 2.36 g/L NaCl [17]. Glochidia of five species (*Villosa constricta*, *Villosa delumbis*, *Elliptio complanata*, *L. fasciola*, and *L. siliquoidea*) had 24-h EC50s ranging from 0.55 to 3.31 g/L NaCl [16]. Juvenile *V. delumbis*, *L. fasciola*, and *L. siliquoidea* had 96-h EC50s ranging from 3.98 to 5.23 g/L NaCl [16]. Valenti et al. [31] reported 24-h EC50s ranging from 2.68 to 3.08 g/L NaCl for glochidia of *L. fasciola*, *Epioblasma capsaeformis*, and *E. brevidens*.

Acute toxicity of salt to other freshwater animals ranges from 1,470 mg Cl/L (2.42 g NaCl/L) for *Daphnia pulex* to 11,940 mg Cl/L (19.68 g NaCl/L) for *Anguilla rostrata* (American eel) [29]. Based on these toxicities, the U.S. EPA set the ambient water quality criterion for chloride at 230 mg Cl/L (0.38 g NaCl/L) for chronic exposures and 860 mg Cl/L (1.42 g NaCl/L) for acute exposures [29]. The Canadian Council of Ministers of the Environment set a lower limit of 120 mg Cl/L for long-term exposure and 640 mg Cl/L for short-term exposure for the protection of aquatic life [32]. Freshwater mussels were not used in the determination of the U.S. EPA's water quality criterion, and the results of the present study and others indicate that the acute criterion may not be protective of all freshwater mussels.

Exposure concerns

Sodium ferrocyanide, the anticaking agent that is used in road deicing salt, is a metal–cyanide complex. The toxicity of cyanide complexes depends largely on the ability of the complex to release free cyanide [12,25,26], and ferrocyanides are among the most stable of all cyanide complexes [11,12]. The mollusk *Chlamys asperrimus* had a 48-h EC50 of 0.0286 mg CN/L for the abnormal development of larvae after exposure to sodium cyanide, whereas exposure to potassium ferricyanide and potassium ferrocyanide resulted in EC50s of 0.128 and 0.686 mg CN/L [25]. These results indicate that the iron–cyanide complexes are less toxic to aquatic organisms than comparable concentrations of free cyanide [12,25]. However, metal–cyanide complexes can be degraded via photolysis by ultraviolet light, thereby releasing free cyanide [11,12].

The U.S. EPA water quality criterion for acute exposure to free cyanide is 0.022 mg/L [27], but runoff from piles of road deicing salts can exceed this concentration. Runoff from piles of road salt at four sites in Maine, USA had free cyanide concentrations of up to 0.096 mg/L and total cyanide concentrations of up to 0.2 mg/L, although free cyanide in runoff from salted roadways at two sites was below detection (<0.01 mg/L) [33]. Runoff at a salt dock in an industrial area had a dissociable cyanide content of 2.9 mg/L out of a total cyanide concentration of 25.6 mg/L [34]. Even at the lowest concentrations of sodium ferrocyanide that are used to prevent salt caking, mass balance calculations predict that the total cyanide concentration in runoff can exceed 0.2 mg/L [33]. Because sunlight can cause complex cyanides such as sodium ferrocyanide to degrade under natural conditions, the amount of free cyanide that may be released from large amounts of salted runoff could pose water quality issues [33,35,36]. Additionally, snow from urban areas may be more acidic and the lower pH of urban snow may affect the toxicity of cyanide in runoff [36].

Although sodium ferrocyanide makes up typically only 0.01% by dry weight of road salt, its presence can result in a measurable increase in cyanide in runoff. Storm sewer outfall from a city that used a mixture of abrasives and 7% salt had a total cyanide concentration of only 0.003 to 0.007 mg/L, whereas roadside snow piles and snow melt from a city using 100% salt contained up to 0.19 mg/L total cyanide [36]. Runoff from the melted snow in a creek in the latter city had total cyanide concentrations that ranged from nondetectable up to 0.045 mg/L [36].

Despite its ability to enter the environment, the cyanide present in deicing salts is most likely a lesser risk to freshwater mussels and other aquatic organisms than chloride. Chloride concentrations spike during the late fall and winter months, particularly in the northeastern and midwestern United States [18,19]. Chloride concentrations in surface water are related to the amount of impervious surface in a watershed [18,19]. A maximum chloride concentration of 2,100 mg/L was observed in a highly urbanized site in Indiana, USA whereas maximum chloride at the reference site was 22 mg/L [19]. Chloride concentrations in four watersheds in Canada ranged from 2.0 to 1,300 mg/L, and 22 to 42% of freshwater mussels species present in these watersheds are at risk from these chloride concentrations [17]. Chloride concentrations as high as 3,050 mg/L have been observed as a result of road deicing, and roadside snowmelt can have chloride concentrations up to 11,800 mg/L [36].

Although chloride concentrations spike in the winter months, chloride may remain elevated into the summer in heavily affected watersheds. Summer chloride concentrations in surface waters in urban and suburban watersheds where road deicing is prevalent can be 100 times greater than chloride concentrations in streams in forested areas [18]. Elevated and protracted chloride concentrations during summer months would coincide with the peak of unionid mussel reproductive activity (i.e., spawning and glochidia release) in northern temperate locations and may adversely impact successful reproduction and recruitment [17].

Cyanide is acutely toxic to aquatic organisms, including the freshwater mussel *V. iris*. In the present study, we found that the effect of cyanide in road salts was negligible compared with the effect of the chloride in the road salts. Because the cyanide in road deicing salts is in a metal–cyanide complex that makes up only a small weight percentage of the salts, it is most likely not a significant threat to aquatic organisms. However, elevated chloride concentrations that result from the use of road deicing salts may present a more relevant threat to aquatic organisms, especially the early life stages of unionid mussels.

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